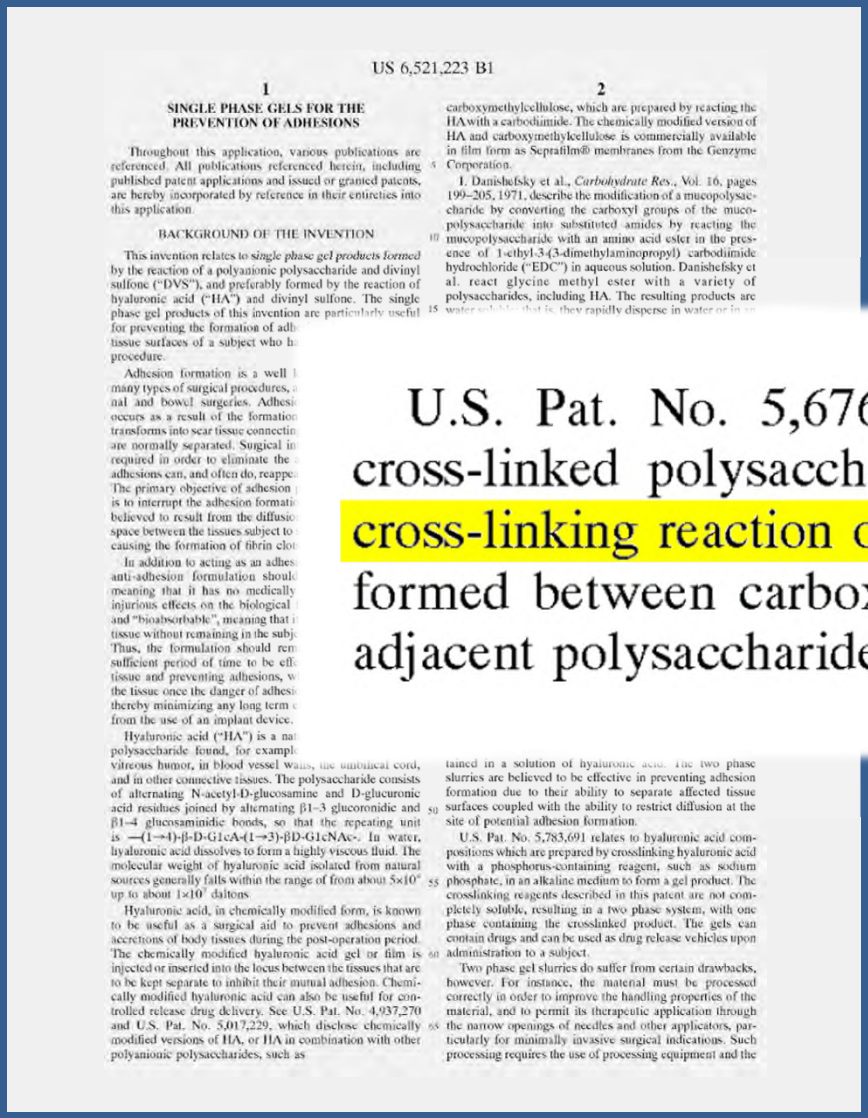
# 6,521,223 B1 Patent, Calias

## Art Describes Crosslinks as Covalent Bonds



US 6,521,223 B1

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**SINGLE PHASE GELS FOR THE PREVENTION OF ADHESIONS**

Throughout this application, various publications are referenced. All publications referenced herein, including published patent applications and issued or granted patents, are hereby incorporated by reference in their entireties into this application.

**BACKGROUND OF THE INVENTION**

This invention relates to *single phase gel products formed* by the reaction of a polyanionic polysaccharide and divinyl sulfone ("DVS"), and preferably formed by the reaction of hyaluronic acid ("HA") and divinyl sulfone. The single phase gel products of this invention are particularly useful for preventing the formation of adh tissue surfaces of a subject who h procedure.

Adhesion formation is a well many types of surgical procedures, nal and bowel surgeries. Adhesi occurs as a result of the formatio transforms into scar tissue connectin are normally separated. Surgical in required in order to eliminate the adhesions can, and often do, reappe The primary objective of adhesion is to interrupt the adhesion formati believed to result from the diffusio space between the tissues subject to causing the formation of fibrin clo

In addition to acting as an adhes anti-adhesion formulation should meaning that it has no medically injurious effects on the biological and "bioabsorbable", meaning that tissue without remaining in the subj Thus, the formulation should rem sufficient period of time to be eff tissue and preventing adhesions, w the tissue once the danger of adhesi thereby minimizing any long term from the use of an implant device.

Hyaluronic acid ("HA") is a na polysaccharide found, for exampl vitreous humor, in blood vessel walls, the umbilical cord, and in other connective tissues. The polysaccharide consists of alternating N-acetyl-D-glucosamine and D-glucuronic acid residues joined by alternating β1→3 glucoronidic and β1→4 glucosaminidic bonds, so that the repeating unit is —(1→4)-β-D-G1cA-(1→3)-βD-G1cNAc-. In water, hyaluronic acid dissolves to form a highly viscous fluid. The molecular weight of hyaluronic acid isolated from natural sources generally falls within the range of from about $5\times10^5$ up to about $1\times10^7$ daltons.

Hyaluronic acid, in chemically modified form, is known to be useful as a surgical aid to prevent adhesions and accretions of body tissues during the post-operation period. The chemically modified hyaluronic acid gel or film is injected or inserted into the locus between the tissues that are to be kept separate to inhibit their mutual adhesion. Chemically modified hyaluronic acid can also be useful for controlled release drug delivery. See U.S. Pat. No. 4,937,270 and U.S. Pat. No. 5,017,229, which disclose chemically modified versions of HA, or HA in combination with other polyanionic polysaccharides, such as

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carboxymethylcellulose, which are prepared by reacting the HA with a carbodiimide. The chemically modified version of HA and carboxymethylcellulose is commercially available in film form as Seprafilm® membranes from the Genzyme Corporation.

I. Danishefsky et al., *Carbohydrate Res.*, Vol. 16, pages 199–205, 1971, describe the modification of a mucopolysaccharide by converting the carboxyl groups of the mucopolysaccharide into substituted amides by reacting the mucopolysaccharide with an amino acid ester in the presence of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride ("EDC") in aqueous solution. Danishefsky et al. react glycine methyl ester with a variety of polysaccharides, including HA. The resulting products are water soluble, that is, they rapidly disperse in water or in an

tained in a solution of hyaluronic acid. The two phase slurries are believed to be effective in preventing adhesion formation due to their ability to separate affected tissue surfaces coupled with the ability to restrict diffusion at the site of potential adhesion formation.

U.S. Pat. No. 5,783,691 relates to hyaluronic acid compositions which are prepared by crosslinking hyaluronic acid with a phosphorus-containing reagent, such as sodium phosphate, in an alkaline medium to form a gel product. The crosslinking reagents described in this patent are not completely soluble, resulting in a two phase system, with one phase containing the crosslinked product. The gels can contain drugs and can be used as drug release vehicles upon administration to a subject.

Two phase gel slurries do suffer from certain drawbacks, however. For instance, the material must be processed correctly in order to improve the handling properties of the material, and to permit its therapeutic application through the narrow openings of needles and other applicators, particularly for minimally invasive surgical indications. Such processing requires the use of processing equipment and the

U.S. Pat. No. 5,676,964 describes the preparation of cross-linked polysaccharides, including HA, wherein the cross-linking reaction occurs as a result of covalent bonds formed between carboxyl groups and hydroxyl groups of adjacent polysaccharide molecules.

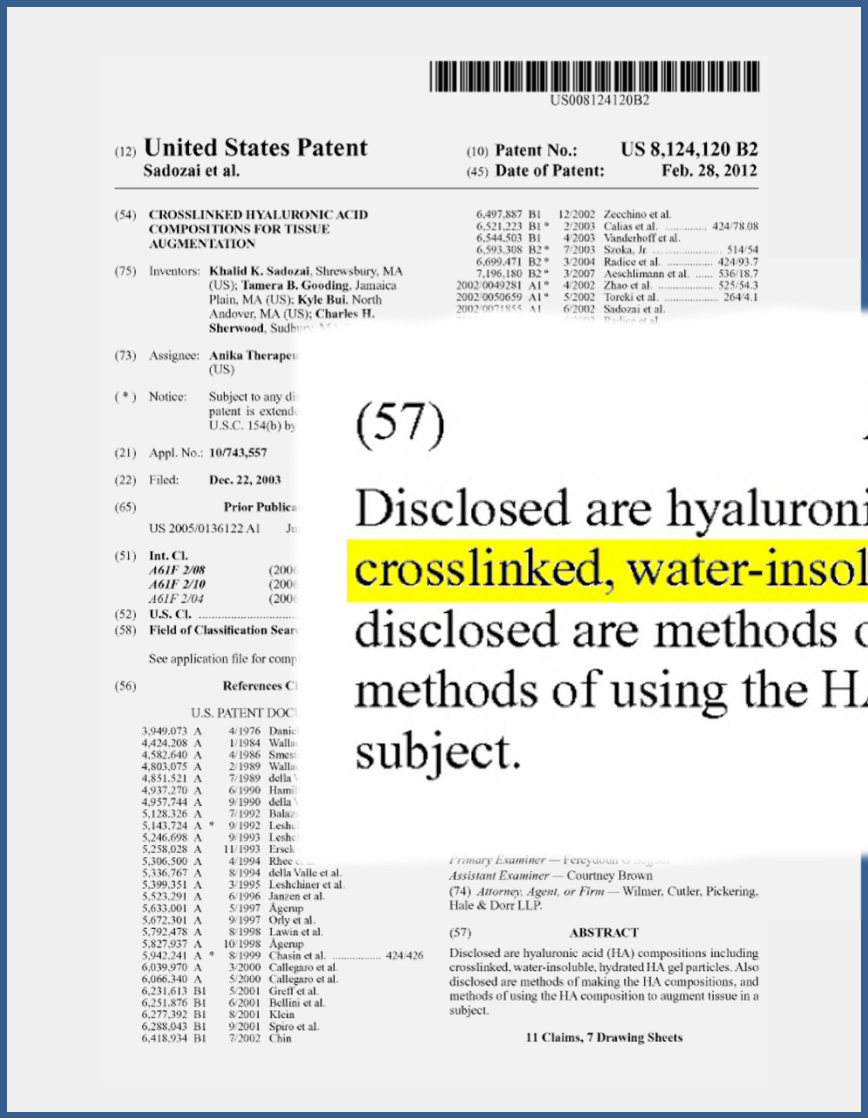
*Source: '223, 2:26-30*

# Second Disputed Term: "Crosslinked"

- Covalently modified
- **Water-insoluble**
- Degree of crosslinking

# 8,124,120 Patent, Sadozai

## Crosslinked HA is Water Insoluble



US008124120B2

(12) **United States Patent**
**Sadozai et al.**

(10) **Patent No.: US 8,124,120 B2**
(45) **Date of Patent: Feb. 28, 2012**

(54) **CROSSLINKED HYALURONIC ACID COMPOSITIONS FOR TISSUE AUGMENTATION**

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(73) Assignee: **Anika Therape** (US)

( * ) Notice: Subject to any di patent is extend U.S.C. 154(b) by

(21) Appl. No.: **10/743,557**

(22) Filed: **Dec. 22, 2003**

(65) **Prior Publica**
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(51) **Int. Cl.**
***A61F 2/08*** (200
***A61F 2/10*** (200
*A61F 2/04* (200

(52) **U.S. Cl.** .....

(58) **Field of Classification Sear**

See application file for comp

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*Primary Examiner* — Fereydoun G
*Assistant Examiner* — Courtney Brown
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(57) **ABSTRACT**

Disclosed are hyaluronic acid (HA) compositions including crosslinked, water-insoluble, hydrated HA gel particles. Also disclosed are methods of making the HA compositions, and methods of using the HA composition to augment tissue in a subject.

**11 Claims, 7 Drawing Sheets**

(57) **ABSTRACT**

Disclosed are hyaluronic acid (HA) compositions including crosslinked, water-insoluble, hydrated HA gel particles. Also disclosed are methods of making the HA compositions, and methods of using the HA composition to augment tissue in a subject.

# 6,521,223 Patent, Calias

## Crosslinked HA is Water Insoluble

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application of shear forces to the material, which in turn can result in a decrease in viscosity (thinning). Two phase materials contain dispersed, heterogeneous particles which tend to plug the narrow openings of such delivery systems. A single phase, homogeneous composition is more useful in minimally invasive surgical applications where devices are introduced into the body through narrow access ports.

It would therefore be highly desirable to formulate a single phase gel solution which is capable of preventing the formation of adhesions, and which can be easily handled and stored for future use, and which possesses the advantageous characteristics of two phase gels.

SUMMARY OF THE INVENTION

The present invention features a cross-linked polyanionic composition which is useful for the prevention of adhesions which can arise as the result of a surgical procedure performed on a subject. The cross-linked composition is prepared by the reaction of the polyanionic polysaccharide with divinyl sulfone. The reaction occurs in an aqueous solution and results in the formation of a gel. The gel solution is neutralized, preferably by acidifying the solution, and a solid is precipitated from the solution. The solid can be pulverized to form a powder, and subsequently rehydrated with water to form a single phase, purified gel having properties suitable for use in anti-adhesion formulations.

In one embodiment, the invention features a method for preparing a single phase gel for use in preventing the formation of surgical adhesions. The gels of this invention are prepared by reacting a polyanionic polysaccharide with divinyl sulfone to form a cross-linked gel. Preferably, the polyanionic polysaccharide is hyaluronic acid or carboxymethyl cellulose, and the molar ratio of divinyl sulfone to polyanionic polysaccharide is from about 0.1:1 to about 1:1, and more preferably from about 0.2:1 to about 0.6:1. The gel is neutralized by the addition of an acidic compound, such as an inorganic acid, typically hydrochloric acid or sulfuric acid, to an aqueous solution of the gel and the cross-linking agent. The gel can be precipitated as a solid, preferably as a powder or fine particles, and stored until it is desired to reconstitute the gel by rehydration of the powder.

Terminal sterilization of the gel can be accomplished by autoclaving the gel, and this procedure does not have any substantial adverse impact on the gel structure. Terminal sterilization is a cost effective method for manufacturing a medical device since it can assure a lower bioburden than aseptic processing, and thereby reduces the risk of infection. Typically, terminal sterilization involves steam autoclaving of aqueous preparations, and either ethylene oxide treatment or high energy bombardment (irradiation or E beam treatment) of the material in solid or dry form.

In one aspect of this embodiment, the properties of the gel are modified by subjecting the gel to heat treatment at a temperature in the range of from about 100° C. to about 150° C. Heat treatment has the effect of modifying the properties of the gel, such as its viscosity. The effect of the heat treatment on specific polymers is generally not predictable in advance, and is based on such factors as the relative degree of cross-linking. Heat treatment of a gel material can be employed to alter the final viscosity of the gel by either causing more polymer to dissolve in solution, which tends to increase the viscosity, or by reducing the molecular weight of the polymer, which tends to reduce the viscosity. Thus, adjustments to the gel viscosity can be easily carried out using this approach.

In another embodiment, the invention features a method for preventing the formation of adhesions by applying the

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cross-linked gel prepared according to the method of this invention to the surface of the tissue which is exposed during a surgical procedure and which is in proximity to the site of the procedure.

The composition can be advantageously applied to the tissue surfaces using non-invasive means, such as by means of endoscopic instruments. Minimally invasive surgical techniques are less traumatic to the patient, more cosmetically appealing, allow faster recovery times, a
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the solution, and precipitating a solid from the solution wherein the polyanionic polysaccharide is hyaluronic acid. In one embodiment, the molar ratio of divinyl sulfone:hy-

The present invention provides a method for preparing a water insoluble biocompatible composition comprising reacting a polyanionic polysaccharide with divinyl sulfone in an aqueous solution to form a gel, neutralizing the pH of the solution, and precipitating a solid from the solution. The polyanionic polysaccharide used may be selected from the group consisting of hyaluronic acid, sodium hyaluronate, potassium hyaluronate, magnesium hyaluronate, calcium hyaluronate, carboxymethylcellulose, carboxymethyl amylose and a mixture of hyaluronic acid and carboxymethylcellulose. In one embodiment of the invention, the solid precipitated from the solution is then rehydrated to form a gel. The invention further provides that the rehydrated gel may then be subjected to heat treatment. In one embodiment, the rehydrated gel is heated to a temperature in the range from about 100° C. to about 150° C.

*Source: '223 patent, 4:45-60*

# WO 96/33751, Debacker

## Crosslinked HA is Water Insoluble



a low degree of crosslinking) dispersed in a liquid phase (that has not undergone crosslinking); said two phases advantageously having been prepared from fibers of Hylan (natural hyaluronic acid modified chemically in situ for the purpose of facilitating its extraction from tissues). It is recommended to use said compositions in many contexts in the medical field.

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Said compositions consist of an injectable suspension whose dispersed phase consists of insoluble fragments of a hydrogel of said highly crosslinked polymer and whose continuous phase consists of an aqueous solution of said polymer and/or of another biocompatible polymer, selected from proteins, polysaccharides and derivatives thereof, slightly crosslinked or not crosslinked.

Exhibit 5
Page 45

VAL0060008

*Source: WO1996033751 A1, 3:14-21 (Translated from French)*

# WO 96/33751, Debacker

## Crosslinked HA is Water Insoluble



In fact, the network of insoluble fragments of the two-phase compositions of the invention is based on molecules of hyaluronic acid joined together by bridges of molecules of crosslinking agent; each of the disaccharide units of said molecules of hyaluronic acid advantageously having between 0.8 and 1 of its hydroxyl functions used in said bridges.

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Moreover, the insoluble fragments of hydrogel of the compositions of the invention can be characterized by other parameters, such as their dry matter content or their optical properties.

In fact, the network of insoluble fragments of the two-phase compositions of the invention is based on molecules of hyaluronic acid joined together by bridges of molecules of crosslinking agent; each of the disaccharide units of said molecules of hyaluronic acid advantageously having between 0.8 and 1 of its hydroxyl functions used in said bridges.

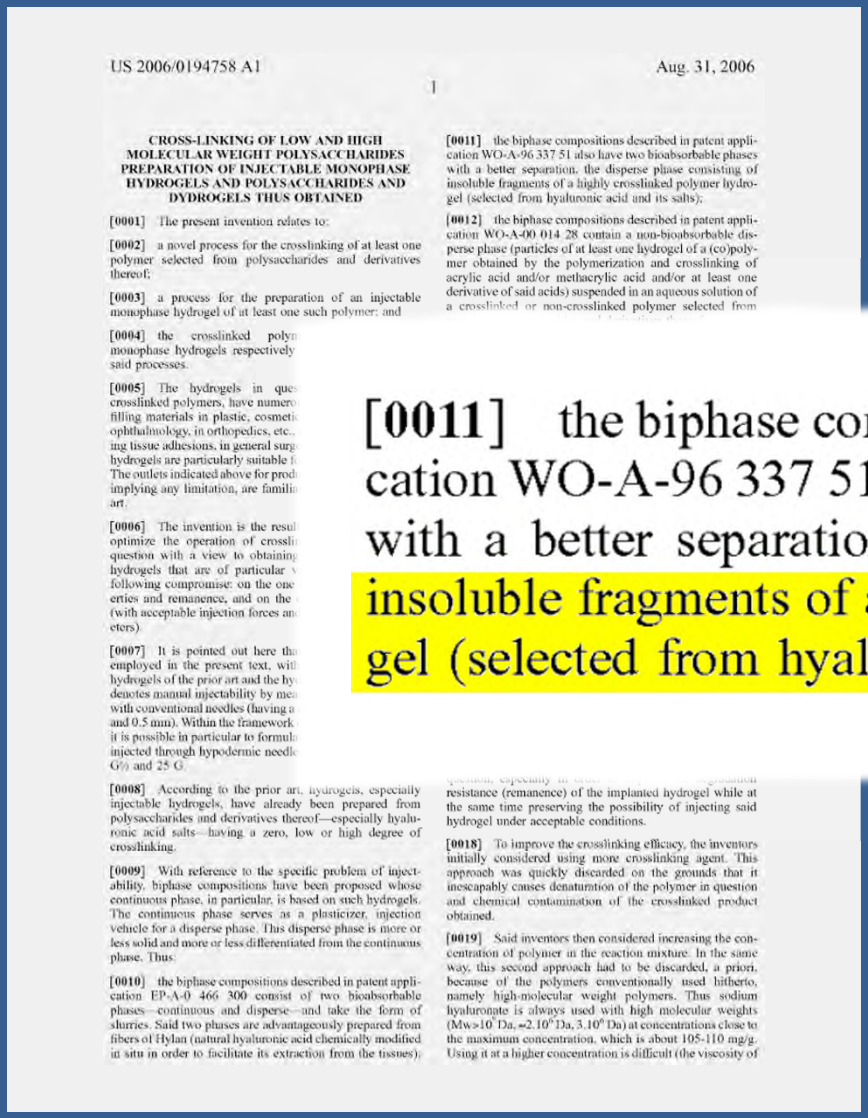
Exhibit 5
Page 50

VAL0060013

*Source: WO1996033751 A1, 8:1-9 (Translated from French)*

# 2006/0194758, Lebreton

## Crosslinked HA is Water Insoluble



US 2006/0194758 A1 Aug. 31, 2006

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**CROSS-LINKING OF LOW AND HIGH MOLECULAR WEIGHT POLYSACCHARIDES PREPARATION OF INJECTABLE MONOPHASE HYDROGELS AND POLYSACCHARIDES AND DYDROGELS THUS OBTAINED**

[0001] The present invention relates to:

[0002] a novel process for the crosslinking of at least one polymer selected from polysaccharides and derivatives thereof;

[0003] a process for the preparation of an injectable monophase hydrogel of at least one such polymer; and

[0004] the crosslinked polym monophase hydrogels respectively said processes.

[0005] The hydrogels in que crosslinked polymers, have numero filling materials in plastic, cosmeti ophthalmology, in orthopedics, etc., ing tissue adhesions, in general surg hydrogels are particularly suitable f The outlets indicated above for prod implying any limitation, are familia art.

[0006] The invention is the resul optimize the operation of crossli question with a view to obtaining hydrogels that are of particular v following compromise: on the one erties and remanence, and on the (with acceptable injection forces an eters).

[0007] It is pointed out here tha employed in the present text, wit hydrogels of the prior art and the hy denotes manual injectability by mea with conventional needles (having a and 0.5 mm). Within the framework it is possible in particular to formul injected through hypodermic needl G½ and 25 G.

[0008] According to the prior art, hydrogels, especially injectable hydrogels, have already been prepared from polysaccharides and derivatives thereof—especially hyaluronic acid salts—having a zero, low or high degree of crosslinking.

[0009] With reference to the specific problem of injectability, biphase compositions have been proposed whose continuous phase, in particular, is based on such hydrogels. The continuous phase serves as a plasticizer, injection vehicle for a disperse phase. This disperse phase is more or less solid and more or less differentiated from the continuous phase. Thus:

[0010] the biphase compositions described in patent application EP-A-0 466 300 consist of two bioabsorbable phases—continuous and disperse—and take the form of slurries. Said two phases are advantageously prepared from fibers of Hylan (natural hyaluronic acid chemically modified in situ in order to facilitate its extraction from the tissues);

[0011] the biphase compositions described in patent application WO-A-96 337 51 also have two bioabsorbable phases with a better separation, the disperse phase consisting of insoluble fragments of a highly crosslinked polymer hydrogel (selected from hyaluronic acid and its salts);

[0012] the biphase compositions described in patent application WO-A-00 014 28 contain a non-bioabsorbable disperse phase (particles of at least one hydrogel of a (co)polymer obtained by the polymerization and crosslinking of acrylic acid and/or methacrylic acid and/or at least one derivative of said acids) suspended in an aqueous solution of a crosslinked or non-crosslinked polymer selected from

resistance (remanence) of the implanted hydrogel while at the same time preserving the possibility of injecting said hydrogel under acceptable conditions.

[0018] To improve the crosslinking efficacy, the inventors initially considered using more crosslinking agent. This approach was quickly discarded on the grounds that it inescapably causes denaturation of the polymer in question and chemical contamination of the crosslinked product obtained.

[0019] Said inventors then considered increasing the concentration of polymer in the reaction mixture. In the same way, this second approach had to be discarded, a priori, because of the polymers conventionally used hitherto, namely high-molecular weight polymers. Thus sodium hyaluronate is always used with high molecular weights ($Mw>10^5$ Da, $\approx 2.10^6$ Da, $3.10^6$ Da) at concentrations close to the maximum concentration, which is about 105-110 mg/g. Using it at a higher concentration is difficult (the viscosity of

[0011] the biphase compositions described in patent application WO-A-96 337 51 also have two bioabsorbable phases with a better separation, the disperse phase consisting of insoluble fragments of a highly crosslinked polymer hydrogel (selected from hyaluronic acid and its salts);

*Source: 2006/0194758, [0011]*

# Second Disputed Term: "Crosslinked"

- Covalently modified
- Water-insoluble
- Degree of crosslinking

# Law on Use of Specification in Claim Construction

"[C]laims "must be read in view of the specification, of which they are a part.'"

*Phillips, 415 F.3d at 1315 (quoting Markman v. Westview Instruments, Inc., 52 F.3d 967, 979 (Fed. Cir. 1995) (en banc))*

"[T]he specification 'is always highly relevant to the claim construction analysis. Usually, it is dispositive; it is the single best guide to the meaning of a disputed term.'"

*Phillips, 415 F.3d at 1315 (quoting Vitronics Corp. v. Conceptronic, Inc., 90 F.3d 1576, 1582 (Fed. Cir. 1996))*

"The context in which a term is used in the asserted claim can be highly instructive," and "[often] provides a firm basis for construing the term."

*Phillips, 415 F.3d at 1314.*